Metabolomic profiling provides new insights into blood-brain barrier regulation

Ogunshola, Omolara O ; Huang, Sheng-Fu

DOI: https://doi.org/10.4103/1673-5374.306077

Metabolomic profiling provides new insights into blood-brain barrier regulation

Sheng-Fu Huang, Omolara O. Ogunshola*

Early blood-brain barrier (BBB) disturbance contributes to many different neurological diseases including Alzheimer's disease, Parkinson's disease and stroke, particularly in the etiology and early stages (Abbott et al., 2010). Although supporting barrier function is a potential strategy to radically improve treatment efficacy and disease outcome, ways to achieve this objective remain elusive. Being a sophisticated system consisting of multicellular interactions, better understanding of how individual cell-specific molecular and metabolic changes modulate the unit responses would provide significant insight. In this regard, we have generated severity-related metabolomic databases to reveal fundamental BBB cell-specific processes likely to occur physiologically and during disease (Huang et al., 2020a). This resource provides new opportunities for future clinical applications.

The BBB plays a critical role in facilitating brain homeostasis (Abbott et al., 2010). It is a complex vascular structure composed of specialized endothelial cells (ECs) that are surrounded by perivascular components namely astrocyte endfeet, pericytes and basal membrane (parenchyma and endothelia membrane). These compartments are intimately linked and interact with one another, resulting in a unique vascular unit. The regulatory mechanisms provided by perivascular components throughout development and adulthood have been mainly described in relation to gene signaling, growth factor secretion and physical support. However, these interactions are interrupted by injury (Abbott et al., 2010). The fact that injury-induced cell-specific responses correlate with BBB impairment (Engelhardt et al., 2015) implies intrinsic adaptation versus susceptibility to a changing environment is a key determinant for whether the associated cells continuously support the BBB or only their own survival.

It is now apparent that ECs themselves undergo disease-specific modulations as well as influence surrounding cells. Our study clearly showed that despite severe injury conditions, and even in absence of other cells, microvascular ECs continuously maintain their physiological metabolomic barrier characteristics (Huang et al., 2020a) rather than activate the adaptive mechanisms observed in ECs from other vascular beds (Li and Carmeliet, 2018). These observations agree with a study showing BBB disruption shifts barrier ECs into a peripheral ECs-like state that compromises brain functions (Munji et al., 2019). Clearly maintaining their specific brain features is intrinsic and this new data further highlights the importance of metabolomic regulation. For instance, during energy depletion and stress autophagy redistributes metabolic resources by liberating required metabolites such as amino and fatty acids, sugars, and nucleosides. Without autophagic processes metabolism is slowly shut down. The reduced metabolic flexibility of brain ECs correlates with the fact that even severe oxygen/glucose deprivation does not activate their autophagy pathway (Engelhardt et al., 2015), in contrast to peripheral EC alterations (Chen et al., 2013). Peripheral ECs also mobilize hypoxia-induced glycolysis and other metabolic alterations (Li and Carmeliet, 2018), whereas brain EC again lack this ability (Huang et al., 2020a). While faithfully and continuously protecting brain homeostasis, the reluctance or inability of brain ECs to metabolically adjust means they are increasingly depleted of critical metabolites. It seems highly feasible that metabolic supplementation to compensate reduced resources would support ECs demands, improve cell tolerance and extend BBB function.

Looking from the brain side of the BBB, understanding the intrinsic metabolic contributions of perivascular cells such as pericytes and astrocytes could also afford substantial gains into ways to modulate this complex unit. The metabolism of brain pericytes has undergone only limited analysis. Recently, however, human placental pericytes were shown to be highly glycolytic and exhibit distinct proliferative and quiescent metabolic characteristics (Cantelmo et al., 2016). Indeed, the relevant study showed that modulation of pericytic metabolism played a key role in tumor vessel stability again highlighting the importance of metabolic crosstalk in vascular functionality. How similar these peripheral pericytes are to those in the brain remains an open question. Intriguingly, hundreds of genes with potentially paracrine-related functions have been identified by RNA-seq in rodent capillary pericytes including many SLC, ATP and ABC transporter families (Chasseigneaux et al., 2018), implying significant communication may occur through this largely unexplored pathway. In contrast, gliovascular modulation has received extensive attention, particularly on the localized function of AC endfeet as local mRNA translation in these processes is critical for synapse modulation and neuron activity (Boulay et al., 2017). By performing cell and ribosome-bound transcriptomics, the "endfeetome" generated reveals that numerous endfeet-localized mRNAs are also BBB related (Boulay et al., 2017). For example, connexin 43 is associated with BBB immune quiescence and angiotensin for BBB maintenance. Furthermore, many transporter mRNAs (including organic anion and amino acid transporters) are also explicitly expressed in astrocyte endfeet (Boulay et al., 2017). Clearly signaling between perivascular cells (both astrocytes and pericytes) and the capillary is more than growth factor stimulation or physical support but involves complex interaction of diverse molecules and metabolites. Whereas transcriptomic and proteomic data reveal potential cellular phenotypic adaptations, the metabolome is the physiological driver that determines overall cell behavior. An intriguing study by Maoz et al. (2018) demonstrated that the BBB proteome and metabolome also modulates surrounding neuronal metabolic activity and impacts the neurovascular unit (NVU) as a whole. Combined mapping of all available "omic" data will undoubtedly provide unprecedented insights into regulation of the intricate BBB and NVU network as a whole.

Which cell initiates metabolic alterations at the BBB? It seems intuitive that ECs, contacting and responsive to the circulatory system, provide the primary signals that modulate metabolic activity in perivascular cells. In agreement, a study showed that transcriptomic signatures could be recapitulated or rejuvenated with acute exposure to aged or young mouse plasma (Chen et al., 2020), underlining that ECs are exquisite sensors of circulatory cues. However direct proof that luminal stimulation alters ECs behaviors to proactively request help from perivascular cells has not been shown. As ECs have low metabolic flexibility (Engelhardt et al., 2015; Huang et al., 2020a) obtaining essential nutrients and support from the more adaptable surrounding perivascular cells would be clever. Indeed we noted that injury-increased abundance of key astrocyte metabolites highly correlated with greater cellular tolerance, and generated significant stores. This led us to propose that astrocyte metabolic shuttling provides essential support for the BBB and

NVU as a whole, similar to the extensive metabolic cooperation known to exist between AC and neurons (Bélanger et al., 2011). Directionality of astrocyte shuttling needs further investigation but we speculate secretion will occur from both apical and basal sides, i.e. towards both neuronal and EC compartments. Whether the level of secretion would be identical or different in either direction is an intriguing question. Indeed astrocytes could be guardians of cells in all brain compartments.

Following up on these ideas, screening our metabolomic data and applying knowledge from previous studies has allowed us to identify beneficial astrocyte-driven metabolites that are potentially shuttled to ECs. We recently showed that glutathione shuttling exists between astrocytes and ECs and is crucial for BBB homeostasis (Huang et al., 2020b), underlining that depletion of a single metabolite can modulate vascular function. With regards to pericytes, a similar conclusion can also be inferred from the above mentioned cancer study (Cantelmo et al., 2016). Thus, exploitation of intrinsic metabolic-driven BBB regulation will undoubtedly provide novel ways to influence brain homeostasis. Above all, these key factors could potentially be "customized" according to different diseases - a concept supported by Nolan et al. (2013) who suggested distinct angiocrine responses from different ECs likely correlate with their specialized metabolic demands. Indeed, targeting EC metabolism has been proposed as a new therapeutic opportunity for drug delivery and functional vascular modulation in cancer treatment (Li and Carmeliet, 2018). Applying a similar strategy could surely facilitate BBB modulation.

BBB researchers frequently use *in vitro* systems to answer complex questions. Our studies, and that of Maoz et al. (2018) using a linked organ-on-chip model, utilize simplified and multicellular culture systems to reveal basic individual cell responses and/or paracellular cross-talk during environmental change, giving extensive insight into adaptation and interactions likely to occur at the BBB *in vivo*. Now we need to build on this knowledge. Combining novel spatial omics with 3D spheroid/organoid disease models more reminiscent of the *in vivo* situation, isolated microvessels and/or brain tissue sections will provide more in-depth information. Particularly untargeted spatial metabolomics coupling technologies, such as matrix-assisted laser desorption/ionization enables metabolites, lipids, and drugs in tissue sections to be localized providing information of cell metabolic behavior and drug metabolism while cells are in contact. These will provide significant insight with the caveats that only a snapshot of the metabolic state at a given point in time is gained and the resolution obtained is still insufficient to observe paracellular shuttling. It is clear that further technological advances will be the key as the data we have presented (Huang et al., 2020a) suggests the EC drive for homeostasis will make assigning metabolic responses to individual cells even in multicellular *in vitro* models difficult, talk less of the *in vivo* situation.

In conclusion, we have demonstrated that cells of the BBB exhibit differential metabolomic profiles during resting and injury conditions that modulate both their own behavior and those of surrounding cells. This metabolomic resource clearly lays important foundations to more fully understand BBB maintenance and regulation. Indeed better understanding of EC metabolic demands and management holds much promise to support barrier health and advance treatment strategies for neuroprotection and drug delivery.

This work was supported by a Swiss National Science Foundation, No. 31003A_170129 (to OOO).

Sheng-Fu Huang, Omolara O. Ogunshola*
Institute for Veterinary Physiology; Zurich Center for Integrative Human Physiology, University of Zurich, Switzerland
***Correspondence to:** Omolara O. Ogunshola, PhD, larao@access.uzh.ch.
https://orcid.org/0000-0002-6409-5470 (Sheng-Fu Huang);
https://orcid.org/0000-0002-1197-4914 (Omolara O. Ogunshola)
Date of submission: August 6, 2020
Date of decision: October 14, 2020
Date of acceptance: November 26, 2020
Date of web publication: January 25, 2021

https://doi.org/10.4103/1673-5374.306077
How to cite this article: *Huang SF, Ogunshola OO (2021) Metabolomic profiling provides new insights into blood-brain barrier regulation. Neural Regen Res 16(9):1786-1787.*
Copyright license agreement: *The Copyright License Agreement has been signed by both authors before publication.*
Plagiarism check: *Checked twice by iThenticate.*
Peer review: *Externally peer reviewed.*

References

Abbott NJ, Patabendige AAK, Dolman DEM, Yusof SR, Begley DJ (2010) Structure and function of the blood-brain barrier. Neurobiol Dis 37:13-25.
Bélanger M, Allaman I, Magistretti PJ (2011) Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. Cell Metab 14:724-738.
Boulay AC, Saubaméa B, Adam N, Chasseigneaux S, Mazaré N, Gilbert A, Bahin M, Bastianelli L, Blugeon C, Perrin S, Pouch J, Ducos B, Le Crom S, Genovesio A, Chrétien F, Declèves X, Laplanche JL, Cohen-Salmon M (2017) Translation in astrocyte distal processes sets molecular heterogeneity at the gliovascular interface. Cell Discov 3:17005.
Cantelmo AR, Conradi LC, Brajic A, Goveia J, Kalucka J, Pircher A, Chaturvedi P, Hol J, Thienpont B, Teuwen LA, Schoors S, Boeckx B, Vriens J, Kuchnio A, Veys K, Cruys B, Finotto L, Treps L, Stav-Noraas TE, Bifari F, et al. (2016) Inhibition of the glycolytic activator PFKFB3 in endothelium induces tumor vessel normalization, impairs metastasis, and improves chemotherapy. Cancer Cell 30:968-985.
Chasseigneaux S, Moraca Y, Cochois-Guégan V, Boulay AC, Gilbert A, Le Crom S, Blugeon C, Firmo C, Cisternino S, Laplanche JL, Curis E, Declèves X, Saubaméa B (2018) Isolation and differential transcriptome of vascular smooth muscle cells and mid-capillary pericytes from the rat brain. Sci Rep 8:12272.
Chen G, Zhang W, Li YP, Ren JG, Xu N, Liu H, Wang FQ, Sun ZJ, Jia J, Zhao YF (2013) Hypoxia-induced autophagy in endothelial cells: a double-edged sword in the progression of infantile haemangioma? Cardiovasc Res 98:437-448.
Chen MB, Yang AC, Yousef H, Lee D, Chen W, Schaum N, Lehallier B, Quake SR, Wyss-Coray T (2020) Brain endothelial cells are exquisite sensors of age-related circulatory cues. Cell Rep 30:4418-4432.e4.
Engelhardt S, Huang SF, Patkar S, Gassmann M, Ogunshola OO (2015) Differential responses of blood-brain barrier associated cells to hypoxia and ischemia: a comparative study. Fluids Barriers CNS 12:4.
Huang SF, Fischer S, Koshkin A, Laczko E, Fischer D, Ogunshola OO (2020a) Cell-specific metabolomic responses to injury: novel insights into blood-brain barrier modulation. Sci Rep 10:7760.
Huang SF, Othman A, Koshkin A, Fischer S, Fischer D, Zamboni N, Ono K, Sawa T, Ogunshola OO (2020b) Astrocyte glutathione maintains endothelial barrier stability. Redox Biol 34:101576.
Li X, Carmeliet P (2018) Targeting angiogenic metabolism in disease. Science 359:1335-1336.
Maoz BM, Herland A, FitzGerald EA, Grevesse T, Vidoudez C, Pacheco AR, Sheehy SP, Park TE, Dauth S, Mannix R, Budnik N, Shores K, Cho A, Nawroth JC, Segrè D, Budnik B, Ingber DE, Parker KK (2018) A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells. Nat Biotechnol 36:865-874.
Munji RN, Soung AL, Weiner GA, Sohet F, Semple BD, Trivedi A, Gimlin K, Kotoda M, Korai M, Aydin S, Batugal A, Cabangcala AC, Schupp PG, Oldham MC, Hashimoto T, Noble-Haeusslein LJ, Daneman R (2019) Profiling the mouse brain endothelial transcriptome in health and disease models reveals a core blood-brain barrier dysfunction module. Nat Neurosci 22:1892-1902.
Nolan DJ, Ginsberg M, Israely E, Palikuqi B, Poulos MG, James D, Ding BS, Schachterle W, Liu Y, Rosenwaks Z, Butler JM, Xiang J, Rafii A, Shido K, Rabbany SY, Elemento O, Rafii S (2013) Molecular signatures of tissue-specific microvascular endothelial cell heterogeneity in organ maintenance and regeneration. Dev Cell 26:204-219.

C-Editors: Zhao M, Wang L; T-Editor: Jia Y